

ABSTRACT OF THE DISCLOSURE

A simple and accurate method for assay of a single-stranded RNA containing a specific nucleic acids sequence in a sample at almost constant temperature by using at  
5 least the following reagents (A) to (I), which comprises a step of adding the reagents (A) to (I) one by one (in any order), in combinations of at least two or all at once and  
a step of measuring a fluorescent signal in the presence  
10 of the reagent (I) at least once after addition of at least the reagents (A) to (H);  
(A) a first single-stranded oligonucleic acid complementary to a sequence neighboring the 5' end of the specific nucleic acids sequence in the single-stranded  
15 RNA,  
(B) a second single-stranded oligo DNA complementary to a 3'-end sequence within the specific nucleic acids sequence,  
(C) an RNA-dependent DNA polymerase,  
20 (D) deoxyribonucleoside triphosphates,  
(E) a third single-stranded oligo DNA having (1) a promoter sequence for a DNA-dependent RNA polymerase, (2) an enhancer sequence for the promoter and (3) a 5'-end sequence within the specific nucleic acids sequence, in  
25 this order from the 5' end,  
(F) a DNA-dependent DNA polymerase,  
(G) a DNA-dependent RNA polymerase,

(H) ribonucleoside triphosphates, and

(I) a fourth single-stranded oligo DNA complementary to the specific nucleic acids sequence which is labeled so that it gives off a measurable fluorescent signal on

5 hybridization with a nucleic acid containing the specific nucleic acids sequence.